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(21) International Application Number: PCT/DK86/00061 (22) International Filing Date: 10 June 1986 (10.06.86) (31) Priority Application Number: 2616/85 (32) Priority Date: 11 June 1985 (11.06.85) (33) Priority Country: DK (71) Applicant (for all designated States except US): NOVO INDUSTRI A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only) : GODTFREDSEN, Sven, Erik [DK/DK]; 15 B, Smedegade, DK-3500 Værløse (DK). ANDRESEN, Otto [DK/DK]; 5, Stenløsevej, DK-3600 Stenløse (DK). INGVORSEN, Kjeld [DK/DK]; 35, Klostergårdsvej, DK-3500 Værløse (DK). YDE, Birgitte [DK/DK]; 33, Østerbrogade, DK-2100 Copenhagen Ø (DK).		(74) Agent: LEHMANN & REE; 26, Frederiksberg Allé, DK-1820 Frederiksberg C. (DK). (81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>
(54) Title: PROCESS FOR PREPARING OPTICALLY ACTIVE, ORGANIC COMPOUNDS (57) Abstract Optically active amino acids or amino acid amides can be prepared by converting an amino nitrile using an enantioselective nitrilase.		

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PROCESS FOR PREPARING OPTICALLY ACTIVE, ORGANIC COMPOUNDS

Background of the invention

The present invention relates to a process for preparing optically active amino acids. More specifically, this invention relates to a process for preparing a single enantiomeric form of an optically active amino acid or amino acid amide which comprises treating an aqueous solution of the enantiomeric mixture of the amino nitrile analog of the amino acid with an enantioselective nitrilase and thereafter recovering the resulting optically active amino acid or amino acid amide.

Optically active amino acids constitute a class of organic compounds of great industrial interest. The naturally occurring amino acids are thus applied industrially on a large scale as food and feed additives and, in recent years, several amino acids not found in nature and in the following referred to as unnatural amino acids have also found extensive use, for example, as constituents in various pharmacological compositions or as intermediates for organic synthesis of optically active compounds.

Due to their molecular structure, most amino acids can occur in two distinct forms differing in respect to the so-called chirality of the amino acid molecule. These two forms of an amino acid which, on the molecular level, are mirror images of one another are usually denoted as the D- and the L-form of the amino acid. Most amino acids found in nature are of the L-configuration and it is essential, therefore, that amino acids used as food and feed additives are also of the L-configuration since the corresponding D-forms or isomers cannot be metabolized by living cells and will interfere with normal cell metabolism and cell function. This ability of the D-amino acids can, however, also be utilized to advantage, for example, by incorporating such unnatural isomers of amino acids into pharmacologically active compounds, the activity of which may be due to or enhanced by a moiety of unnatural chirality in its molecular structure. In such instances, it is essential that the amino acid used only is of the unnatural configuration since the presence of

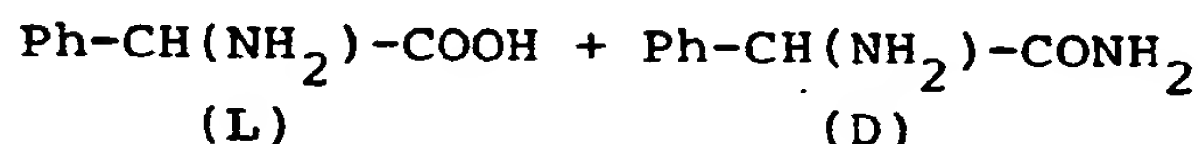
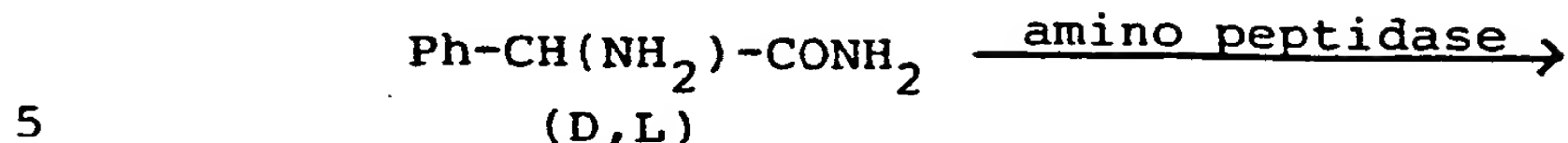
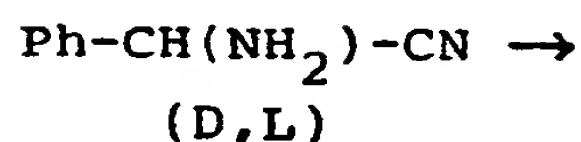
molecular species carrying the natural configuration will, in such instances, exert a deleterious effect on the biological activity of the compound in question.

Because of the wide use of natural as well as of 5 unnatural amino acids it is, in general, highly desirable to have available optically pure, i.e., enantiomerically pure, amino acids of the natural as well as of the unnatural configuration for a wide variety of industrial applications of amino acids while, on the contrary, mixtures of the D- and 10 L-forms of amino acids, the so-called racemates, are of limited industrial interest only.

The desire to provide an excess amount of one enantiomer in preparations of amino acids is reflected in the methods currently used for industrial production of such 15 compounds. Most amino acids used as food and feed additives are thus produced by microbial fermentations which, due to the very nature of the microorganisms, give rise solely to amino acids of the natural configuration. Also, enzymes derived from microorganisms or other living matter have been 20 used for the production of amino acids which, in such instances, derive their chirality from the chirality of the applied enzyme.

An example of an enzymatic method which has been used for preparation of optically active amino acids is 25 described in U.S. patent specifications Nos. 4,080,259 and 3,971,700. The process disclosed in these patents can be illustrated in the following Scheme 1:

3



wherein Ph represents, for example, phenyl.

As indicated, an enzyme, i.e., an amino acid
 10 amidase, a so-called amino peptidase, is utilized for converting amino acid amides into the corresponding amino acids. As appear from Scheme 1, the amino acid amides used in the process illustrated are made available by chemical synthesis from achirale starting materials via racemates of amino acid
 15 nitriles, the consequence being that the amino acid amides used in the process described are racemic mixtures. The enzyme used in the process is, however, chirale and, therefore, capable of distinguishing between the two isomeric forms of the amino acid amide. As a consequence, the amino
 20 acids generated in the course of the amino peptidase catalyzed reaction are of the L-configuration while the amino acid amides remaining in the reaction mixture after completion of the enzymatic conversion are of the D-configuration. These two, chemically distinct species, can be
 25 separated by conventional methods and the enantiomeric pure amino acid amides thus obtained can subsequently be hydrolyzed by chemical means to provide optically pure D-amino acids. The method disclosed in the above U.S. patent specifications serves, therefore, as a means for the
 30 preparation of optically pure L- as well as D-amino acids.

The use of enzymes for the conversion of amino nitriles into the corresponding amino acid amides is a feasible process which, however, does not so far offer any

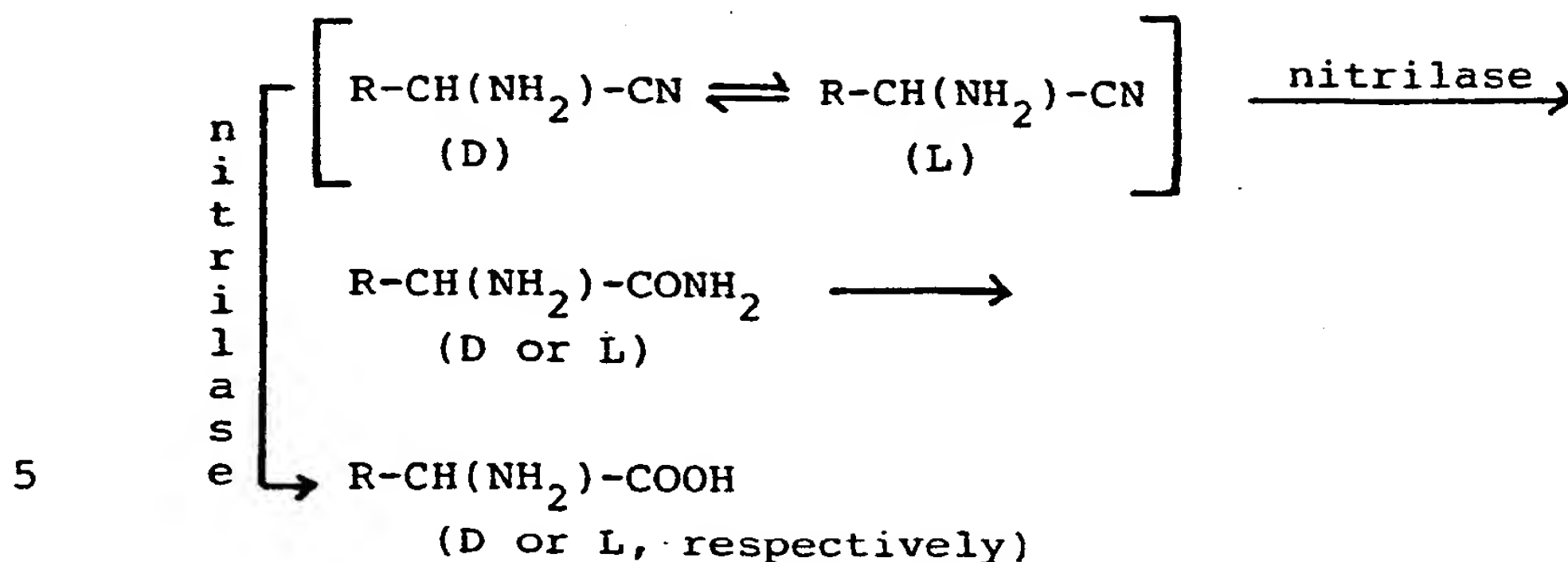
advantages as compared to the chemical hydrolysis. As described by Jallageas and co-workers in Advances in Biochemical Engineering, 14 (1980), 1 - 32, enzymatic conversion of amino nitriles give rise to a mixture of D-amino acid amides and L-amino acids. Accordingly, this enzymatic process suffers from disadvantages similar to those of the chemical conversion of amino nitriles, i.e., only half of the racemic starting material is converted into the desired amino acid which has to be separated from the D-amino acid amide generated in the course of the reaction.

Summary of the invention

The process of the present invention is characterized in that the conversion of amino nitriles into the corresponding amino acids or amino acid amides is effected with a nitrilase which preferentially converts one of the two enantiomeric forms of the amino nitrile into the corresponding amino acid or amino acid amide.

The process of the invention is based on the surprising observation that enantioselective nitrilases can be found and used under conditions which serves to generate, from racemic amino nitriles, amino acids and amino acid amides containing an excess of one enantiomer. Even though it is preferred only to obtain the desired compound, normally a mixture containing an excess of the desired compound is obtained. The essential features of this invention are illustrated in the following Scheme 2:

5



wherein R is as defined below.

As indicated, the amino nitrile used as starting material is present in the form of a mixture of the D- and L-form of the amino nitrile, for example, in equal amounts. The nitrilase applied according to the process of this invention converts, however, preferentially one of these two enantiomers into either the corresponding amino acid amide or, directly, into the corresponding amino acid. As a consequence, the reaction mixture obtained by the enzymatic conversion contains an excess of one of the two enantiomers, i.e., the amino acid or the amino acid amide. Since, as also indicated in Scheme 2, conversion of one enantiomer of the amino nitrile into the other enantiomer will occur concomitantly with the enzymatic, preferential conversion of one of the enantiomers of the amino nitrile, the entire racemic mixture of amino nitriles can, according to this invention, be converted into a reaction mixture containing an excess of one enantiomer of an amino acid or an amino acid amide.

The compounds which can be prepared by the process of this invention can be represented by the general formula I



wherein R represents indolyl; benzyl; benzyloxy; lower alkyl; all optionally substituted by hydroxy, mercapto, amino, halogen, phenyl, phenoxy, benzyl or lower alkylthio; or

phenyl optionally substituted by one or more of the following substituents: hydroxy, amino, halogen, carboxy or lower alkoxy; and X represents hydroxy or amino; or salts thereof.

Hence, the starting material is an amino nitrile of the general formula II



wherein R is as defined above, or a salt thereof..

Examples of the substituent designated R are as follows: methyl, isopropyl, secondary butyl, phenyl, p-hydroxyphenyl, benzyl, 1-hydroxyethyl, mercaptomethyl, methylthiomethyl, benzyloxy and phenoxymethyl. Preferably R is indolyl or benzyl optionally substituted by one or more of the following groups: hydroxy, amino and/or lower alkoxy.

Herein the term lower alkyl designates alkyl containing less than 8, preferably less than 5, carbon atoms. Similarly, lower alkoxy contains less than 8, preferably less than 5, carbon atoms.

The enzymatic process may, according to this invention, be carried out, for example, in a batch-wise fashion by stirring a mixture of the nitrilase and the amino nitrile in an aqueous solution under control of the pH value and temperature of the reaction mixture. The reaction temperature may be between the freezing point of the reaction medium and about 65°C, preferably between 20 and 45°C, most preferred about 37°C. If desired, organic solvents can be utilized to increase the solubility of the reactants, such solvents being, for example, alcohols such as ethanol, methanol, isopropanol or tertiary butanol or organic solvents such as dioxane, N,N-dimethylformamide, dimethylsulfoxide or hexamethylphosphorous triamide. The reaction may also be carried out in a two-phase system using a suspension of reactants or two immiscible solvents like, for example, water and a hydrocarbon such as hexane or cyclohexane.

The nitrilase applied in the process of this invention may be a purified enzyme, a crude enzyme solution, microbial cells exhibiting the desired activity or a

homogenate of cells. If required, the enzyme may be used in an immobilized state or in a chemically modified form to ensure a good stability and reactivity of the applied enzyme under the reaction conditions utilized.

5 The process of this invention can be carried out at neutral or at an alkaline pH value to ensure rapid interconversion of one of the two enantiomeric forms into the other of the two enantiomeric forms of the amino nitriles used as starting material in the enzymatic process. This intercon-
10 version can also take place at a pH value below 7 or it can be ensured by applying an amino nitrile racemase. Hence, preferentially, the pH value is from about 6 to about 13.

 As mentioned above the nitrilases used by the process of this invention are enzymes exhibiting a different
15 activity towards the two enantiomeric forms of amino nitriles. Preferably, nitrilases exhibiting a strong selectivity towards one of these enantiomers are used since it is usually desired that the amino acids or amino acid amides prepared by the process of this invention contain a
20 large excess of one of the two enantiomers. In a preferred embodiment of this invention, the excess of one of the two enantiomeric forms of the amino acid or amino acid amide is greater than 25%. Accordingly, it is preferable to test nitrilases prior to use for conversion of a given amino
25 nitrile. This test can be carried out, for example, by exposing the amino nitrile in question to the enzyme preparation and by, subsequently, isolating, after conversion of a small amount of the amino nitrile, the amino acid amide and/or amino acid formed, for example, by high pressure
30 liquid chromatography, and by analyzing the optical purity of the isolated compounds. Preferably, this test is carried out at various degrees of conversion of the applied amino nitrile.

 The enzymes for use in the process of this
35 invention may be isolated from microorganisms, plants or animals. Preferably, however, enzymes of microbial origin are utilized, such microorganisms being bacteria, fungi or other microorganisms.

- Examples of microbial species producing nitrilases are as follows: Species of Pseudomonas, Gluconobacter, Acetobacter, Achromobacter, Acinetobacter, Citrobacter, Enterobacter, Erwinia, Escherichia, Klebsiella, Proteus,
 5 Serratia, Yersinia, Aeromonas, Vibrio, Staphylococcus, Streptococcus, Clostridium, Leuconostoc, Cellulomonas, Microbacterium, Propionibacterium, Mycobacterium, Streptomyces, Chaetomella, Septoria, Diplodia, Phoma, Conothyrium, Myrothecium, Pestalotia, Melanconium, Epicoccum,
 10 Penicillium, Aspergillus, Sepedonium, Fusidium, Oidiodendron, Cephalosporium, Scopulariopsis, Paecilomyces, Verticillium, Tricothecium, Pullularia, Monotospora, Cladosporium, Helminthosporium, Chrysosporium, Rhodotorula, Kloeckera, Geotrichum and preferably Fusarium, Agrobacterium,
 15 Arthrobacter, Alcaligenes, Shigella, Peptococcaceae, Pseudomonadaceae, Cytophaga, Bacteroidaceae, Butyrivitrio, Selenomonas, Zymomonas, Chromobacterium, Flavobacterium, Micrococcus, Pediococcus, Bacillus, Lactobacillus, Brevibacterium, Thermus, Corynebacterium, Hyphomicrobium,
 20 Bacteridium, Actinomycetales, Rhizopus, Mucor, Candida, Saccharomyces, Nocardia, Rhodococcus, Stenphylium and Toryloopsis, strains of Agrobacterium radiobacter, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Corynebacterium nitrilophilus, Corynebacterium
 25 pseudodiphtheriticum, Nocardia rhodochrous, Escherichia coli, Neurospora crassa, Lathyrus sylvestris, Lathyrus odoratus, Vicia villosa, strain A4 (deposited at Laboratory of Microbiology (hereinafter designated LMD), the Netherlands, under No. LMD 79.2), strains N-771, N-774 and N-775
 30 (deposited at Fermentation Research Institute (hereinafter designated FRI), Japan, under No. 4445, 4446 and 4447, respectively) and strains R 332 (deposited at Centraalbureau voor Schimmelcultures (hereinafter designated CBS), the Netherlands), R 340 (CBS No. 495.74), R 341 (CBS No. 496.74),
 35 A 111 (CBS No. 497.74), B 222 (CBS No. 498.74), A 112, A 13, A 141, A 142, B 211, B 212, B 221, C 211 (CBS No. 499.74), R

21, R 22, R 311, R 312 (CBS No. 717.73) and R 331 stated in Table I in U.S. patent specification No. 4,001,081 which is hereby incorporated by reference.

The desired amino acid amide or amino acid is
5 isolated from the reaction mixture in a manner known per se, for example, by precipitation, optionally after adjustment of the acidity, or evaporation.

The features disclosed in the foregoing description and in the following examples and claims may, both separately
10 and in any combination thereof, be material for realising the invention in diverse forms thereof.

The process of this invention will be further illustrated by the following examples which, however, are not to be construed as limiting. The examples illustrate some
15 preferred embodiments.

Example 1

Preparation of optically active L-leucine amide

A preparation of an enantioselective aminonitrile hydratase was prepared by cultivating nitrilase producing
20 strain No. 311 (deposited in May 1986 at the National Collection of Industrial Bacteria (NCIB) under the number NCIB 12256) in a modified M9 medium (c.f. Maniatis et. al., Molecular Cloning, A Laboratory Manual, CSH, 1982) containing 1% glucose, 0.05% yeast extract and 0.5% acetonitrile as
25 substitute for ammonium chloride. The biomass generated was harvested after three days of growth at 37°C, washed thoroughly with phosphate buffer (0.1 M, pH 7) and finally stored as a suspension in said buffer. This suspension was used as the enzyme solution in the following examples.

30 A solution of racemic leucine aminonitrile was prepared in the following manner:

Ammonium chloride (0.032 mol) in 5.5 ml of water was added at room temperature to a solution of 3-methylbutanal (0.031 mol) in 2.2 ml of water. After 30 minutes, the
35 mixture was cooled to 0°C and a solution of sodium cyanide (0.031 mol) was added. The resulting mixture was then stirred for one hour at 0°C and then for 12 hours at room

temperature. Finally, the solution was diluted with phosphate buffer (0.1 M, pH 7) to a final concentration of the aminonitrile of 120 mM.

Enzymatic hydrolysis of the aminonitrile was subsequently performed by adding 0.1 ml of enzyme solution per 0.3 ml of the solution of the aminonitrile, stirring of the resulting mixture for 1 hour, removing the enzyme by centrifugation, and finally adsorbing the product and eluting it from an ion-exchange resin. The amide isolated in this fashion was found to contain an enantiomeric excess of the L-amide of 40%.

Example 2

Preparation of optically active L-leucine

A solution of leucine amino nitrile was made and treated with the enzyme solution described above in a manner analogous to that described in Example 1. At intervals during the enzymatic hydrolysis, the enzyme was removed by centrifugation after which pH of the reaction mixture was adjusted to 11 by addition of a 2 M sodium hydroxide solution. After 15 minutes, the pH of the reaction mixture was adjusted to its initial value and mixed with the biocatalyst. This procedure was carried out 5 times during a total reaction period of 6 hours after which conversion of the aminonitrile into the amino acid was complete as determined by thin layer chromatography. The amino acid was then isolated by ion-exchange chromatography and found to contain an enantiomeric excess of 35%.

Example 3

Preparation of optically active L-valine amide

L-valine amide was prepared from isobutyraldehyde in a manner analogous to that described in Example 1. The enantiomeric excess of the L-amide in the reaction mixture was found to be 35%.

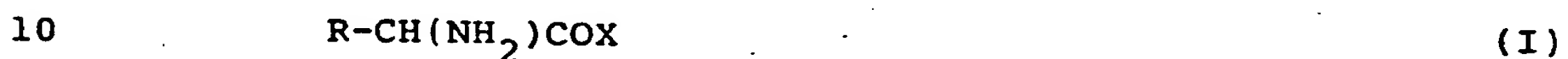
Example 4Preparation of optically active L-valine

L-valine was prepared from isobutyraldehyde in a manner analogous to that described in Example 2. The 5 enantiomeric excess of the L-amino acid was found to be 30%.

C L A I M S

1. A process for preparing an amino acid or amino acid amide which comprises treating a solution of an enantiomeric mixture of the corresponding amino nitrile with
5 an enantioselective nitrilase and subsequently recovering the resulting optically active amino acid or amino acid amide.

2. A process, according to Claim 1, characterized in preparing optically active amino acids or amino acid amides of the general formula I



wherein R represents indolyl; benzyl; benzyloxy; lower alkyl optionally substituted by hydroxy, mercapto, amino, halogen, phenyl, phenoxy, benzyl or lower alkylthio; or phenyl optionally substituted by one or more of the following sub-
15 stituents: hydroxy, amino, halogen, carboxy or lower alkoxy; and X represents hydroxy or amino; or salts thereof.

3. A process according to Claim 1 or 2, characterized in preparing an amino acid or amino acid amide of L-configuration.

20 4. A process according to any one of the preceding claims, characterized in preparing the enantiomeric amino acid or amino acid amide in an excess of at least 25%.

5. A process according to any one of the preceding claims, characterized in effecting the treatment at a pH
25 value from about 6 to about 13.

6. A process according to any one of the preceding claims, characterized in that the conversion is effected in the presence of an amino nitrile racemase.

7. A process according to any one of the preceding
30 claims, characterized in using a reaction temperature of from about 20 to about 45°C, preferably about 37°C.

8. A process according to any one of the preceding claims, characterized in that the conversion is effected in an aqueous medium optionally containing an alcohol, dioxane,
35 N,N-dimethylformamide, dimethylsulfoxide or hexamethylphosphorous triamide.

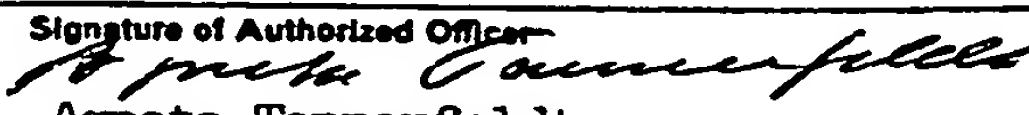
9. A process, according to any of the preceding claims, characterized in using a nitrilase of microbial origin, preferably of bacterial origin.

10. A process according to claim 9 characterized in using an aminonitrile hydratase with enzymatic properties substantially identical with those of the aminonitrile hydrotase obtained by cultivation of Strain No. 311 deposited at the National Collection of Industrial Bacteria (NCIB) under number NCIB 12256 or a mutant thereof.

10 11. The use of an enantioselective nitrilase for the conversion of an aminonitrile into the corresponding optically active amino acid or amino acid amide.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK86/00061

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC 4 <div style="text-align: center; font-family: monospace; font-size: 1.2em;">C 12 P 41/00</div>		
II. FIELDS SEARCHED		
Minimum Documentation Searched 7		
Classification System	Classification Symbols	
IPC	C 07 B 19/02; C 12 N 9/78, /80; C 12 P 13/00-/14, /20-/24, 41/00	
US Cl	195: 2, 30, 50; 435: 106-110, 113 - 116, 128, 129, 227, 228	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
SE, NO, DK, FI classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	FR, A, 2 447 359 (AGENCE NATIONALE DE VALORISATION DE LA RECHERCHE) 22 August 1980	1-3,5,9,10,11
X	Chemical Abstracts, Vol 96 (1982) abstract No 160 701q, Adv Biotechnol 6th, 1980, 3, 227-33 (Fr)	1-3,9,11
A	EP, A, 0 093 782 (YAMADA) 16 November 1983	
A	US, A, 3 940 316 (AGENCE NATIONALE DE VALORISATION DE LA RECHERCHE) 24 February 1976	
A	US, A, 4 001 081 (AGENCE NATIONALE DE VALORISATION DE LA RECHERCHE) 4 January 1977	
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: 10</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
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International Searching Authority	Signature of Authorized Officer	
Swedish Patent Office	 Agneta Tannerfeldt	

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BAD ORIGINAL

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US19900632022 19901221; US19940277775 19940720

CPY - NIHA

DC - B05 D13 D16 D21 E14 E16 E19

DR - 1210-P

FS - CPI

IC - C12P13/06 ; C12P13/08 ; C12P41/00 ; C12R1/01

IN - FURUHASHI K; MIURA A; TAKAHASHI O; WAKAMOTO A

MC - B10-B02J D05-C01 E10-B02D6

M2 - [01] H1 H100 H181 J0 J011 J1 J171 M280 M312 M321 M331 M340 M342 M349
M381 M391 M416 M620 M720 M800 M903 M904 M910 N131 N184 N235 N309 N341
N362 N512 Q233; R07395-P

M3 - [01] H1 H100 H181 J0 J011 J1 J171 M280 M312 M321 M331 M340 M342 M349
M381 M391 M416 M620 M720 M800 M903 M904 M910 N131 N184 N235 N309 N341
N362 N512 Q233; R07395-P

PA - (NIHA) NIPPON MINING CO

PN - JP4079894 A 19920313 DW199217 003pp

- US5587303 A 19961224 DW199706 C12P13/08 018pp

PR - JP19900191676 19900719; JP19880052694 19880308; JP19880052695 19880308;
JP19880055781 19880309; JP19900155661 19900614; JP19900191677 19900719

XA - C1992-063925

XIC - C12P-013/06 ; C12P-013/08 ; C12P-041/00 ; C12R-001/01

XR - 1989-265591 1991-189945 1992-137927

AB - J04079894 D-Alanine is prepd. by adding Rhodococcus sp. hydrolysing
nitrile cpd., or its prepd. substance, to racemic 2-aminopropionitrile.

- USE/ADVANTAGE - High optically purity D-alanine can be obtd. from
racemic 2-aminopropionitrile, directly in high yield.

- In an example, 100 ml of medium (sucrose 1%, Na2HPO4.12H2O 0.25%,
KH2PO4 0.2%, MgSO4.7H2O 0.05%, FeSO4.7H2O 0.003%, CaCl2.2H2O 0.006%,
isobutyronitrile 0.5%, pH 7.2) was charged into 500 ml vol. flask. To
this, 4 ml of Rhodococcus rhodochrous PA-34 strain cultured in
Nutrient Broth No.2 (2.5% : Difco Co.) and glucose (1%) contained
medium (pH 7.5), was inoculated and cultured at 30 deg.C for 24 hours.
Obtained cultured soln. was centrifuged at 10000G for 10 min..
2-Amino-propionitrile (7.6 mg) was added, and shaken at 30 deg.C for
60 min.. 0.99 mg of D-alanine (68.1% ee) was produced. (Dwg.0.0)

CN - R07395-P

IW - HIGH OPTICAL PURE ALANINE PREPARATION ADD RHODOCOCCUS SPECIES RACEMIC
AMINO PROPIONITRILE

IKW - HIGH OPTICAL PURE ALANINE PREPARATION ADD RHODOCOCCUS SPECIES RACEMIC
AMINO PROPIONITRILE

INW - FURUHASHI K; MIURA A; TAKAHASHI O; WAKAMOTO A

NC - 002

OPD - 1988-03-08

ORD - 1992-03-13

PAW - (NIHA) NIPPON MINING CO

TI - High optical purity D-alanine prepn. - by adding rhodococcus sp. to
racemic 2-amino:propionitrile

USAB- US5587303 A process for producing an optically active alpha-amino
acid in L-configuration represented by formula (I'):

- from the alpha-aminonitrile compound represented by formula (I):
- R= isopropyl, butyl, or isobutyl group, which process comprises the steps of:
 - (a) contacting a microorganism having stereospecific nitrilase activity and selected from the group consisting of: Nocardiosis sp. B96-47, FERM BP-2423; Nocardiosis sp. A10-12, FERM BP-2422; Bacillus sp. B9-40, FERM BP-3992; and Bacillus sp. A9-1, FERM BP-3991 and the alpha-aminonitrile in a reaction medium of an ammoniacal buffer solution at a pH 8-12 to obtain the optically active alpha-amino acid in L-configuration; and
 - (b) recovering the optically active alpha-amino acid in L-configuration thus obtained from the reaction medium.
- (Dwg.0/0)

(C) BIOSIS / BIOSIS

XP-002165676

AN - PREV199192083701

TI - ENZYMATIC HYDROLYSIS OF RACEMIC PHENYLALANINAMIDE WITH PRONASE
IMMOBILIZED ON KETONIC POLYMER

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AB - In the transformation of an aldehyde into an .alpha.-amino acid (Strecker's reaction or related), we try to enantioselectively hydrolyse the .alpha.-aminonitrile intermediate using a catalyst obtained by the immobilization of pronase on chemically reactive polymer presenting piperidone groups. This catalyst should be able to simultaneously hydrate the .alpha.-aminonitrile and to enantioselectively hydrolyse the .alpha.-aminoamide obtained. First of all, we define the essential parameters of this reaction (pH, substrat concentration, catalyst mass) by a kinetic study. These parameters determine the activity of immobilized pronase in the enantioselective hydrolysis of DL-phenylalaninamide.

